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DIFFERENCES IN CRANIOFACIAL SHAPE AMONG A/J AND C57BL/6J MICE AND THEIR F1 CROSSES

by

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Submitted to the Graduate Faculty of the School of
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INTRODUCTION

Many authors have found relationships between various craniofacial measurements and the occurrence of cleft lip (CL) in humans.[1-9] Other authors have found similar relationships in mice.[10-14] Although it is widely recognized that a relationship exists between oral clefting and facial shape, this relationship is poorly understood.[15] CL occurrence has long been thought the result of a multifactorial threshold mode of inheritance.[16] More recent data from studies of CL in mice suggest that there is also a maternal effect in the incidence of CL in offspring.[17-24] The dried skulls of two strains of mice will be used in this experiment, as well as two versions of their offspring (F-1): A/J strain, which has a high spontaneous rate of CL;[12, 17, 21] C57BL/6J strain, which has virtually no CL;[12, 17, 25] offspring in which A/J is the mother and C57BL/6J is the father (AB6F1/J) and the reciprocal offspring with C57BL/6J as the mother and A/J as the father (B6AF1/J). The skulls will be digitally photographed and several craniofacial landmarks will be digitally mapped on each image. From these landmarks, sets of ratios correlating to various shapes of the craniofacial complex will be compared among the four groups. The null hypothesis (H_o) for this research experiment is that there will be no differences in craniofacial shape between the two F-1 generations of the two strains of mice, since both groups will be genetically identical. An alternate hypothesis (H_A) is that the F-1 mice will show differences in craniofacial shape, depending on the maternal strain.

REVIEW OF LITERATURE

LIP AND PALATAL DEVELOPMENT

The development of the lip and palate is a complex sequence of events. It is generally accepted that the cranial neural crest cells give rise to most of the facial tissues, including the skeletal and connective tissue components of the lip and palate.[26] The neural crest cells migrate from the embryonic neural fold to develop into regions of growth prominences such as the medial nasal, lateral nasal, and maxillary prominences. These prominences normally meet with each other and fuse, thus separating the nasal cavity from the oral cavity.[27]

The primary palate is the embryonic structure that separates the oral and nasal cavities in the anterior region. It is the precursor to portions of the upper lip, maxillary alveolar process containing the four incisors, and the hard palate anterior to the nasopalatine canal. It develops from the fusion of the epithelia of the medial and lateral nasal processes below the nasal pit. When these two processes fail to completely fuse, a cleft of the lip results.[26] Clefts of the lip, with or without cleft palate (CL(P)) are the most common facial malformations.[28]

The secondary palate is the embryonic structure that gives rise to the soft palate, and the hard palate posterior to the nasopalatine canal. The palatal shelves are extensions of the maxillary processes, and early in development grow downward on each side of the tongue. During development, the shelves go through a process of elevation to a position above the tongue, at which point the medial edges of the shelves come into contact and fuse with each other.[26] When the processes fail to fuse, cleft palate results.

MECHANISMS OF FAILURE

Given the complexity of development, it is no surprise that there are several proposed mechanisms of failure leading to a resultant CL(P). Each of the processes involved in attaining the normal structures may be influenced by genetic factors such as size and shape of growth, or epithelial activity; environmental factors such as nutrition, drugs, or oxygen concentration; or both.[27] A more detailed review of some of these factors will follow.

Multifactorial Threshold Hypothesis

As already described, there are many factors that can contribute to the incidence of CL(P). However, not *all* of these factors are observed in every instance of the anomaly. Falconer[29] first developed the concept of a multifactorial basis for some common diseases. Fraser[30] explains the multifactorial threshold hypothesis as an individual's liability for a disease (such as CL(P)) coming from the sum effect of several different genetic and environmental factors. Once the effect of all the additive liabilities crosses a certain threshold, the disease or anomaly occurs. He also clarifies that the term "multifactorial" should be used without regard to the nature of the genetic factor(s).

ANIMAL STUDIES

Face Shape and Cleft Lip Relationship

In 1968 Trasler[12] postulated that there is a relationship of embryonic facial shape to CL in mice after studying two different mice stains, A/J and C57BL/6J. The A/J strain has a spontaneous clefting frequency of about 10-14%,[12, 17, 21] whereas the C57BL/6J strain has virtually no clefting.[12, 17, 25] Based upon her observations of the differences in

embryonic facial development between the two inbred mouse strains, she hypothesized that these differences, and the way in which they developed, made it more likely for the A/J strain to have CL than the C57BL/6J strain. In discussing whether these factors may also be involved in the development of CL in humans, she noted how similar the facial development of mice was to that of humans at this stage of embryogenesis. She concluded that differences in embryonic facial development in humans as well as mice might be a contributing factor towards the development of CL. Johnston *et al.*[26] later concurred that CL in the A/J mouse was very similar to the uncomplicated form of human CL in a number of ways, including etiology.

To test the hypothesis that embryonic face shape is a causal factor in genetic predisposition to cleft lip in mice, Juriloff and Trasler[11] conducted an experiment using three different strains of mice. The three strains were all derived from the same parent strains, and were selected based on their susceptibility to facial clefting after maternal exposure to 6-aminonicotinamide (6AN) given on day 9.5 of gestation. Line L was selected for lateral cleft lip, line M was selected for median facial clefts and line C had been maintained without selection and was used as a control. Non-treated embryos were collected at day 10 of gestation, and those in the late oblong to crescent stages of embryonic development were photographed and measured. The distance between the nasal pits was significantly smaller ($p \leq 0.05$) in line L than in lines C or M, which was predicted by their hypothesis. The mean angle of divergence of the medial nasal processes was smallest in line L and greatest in line M, also as predicted by their hypothesis. Though they acknowledge that there are other factors that likely contribute to

this threshold trait of CL, their results supported the hypothesis that embryonic facial shape could be an underlying factor.

The embryonic face shape hypothesis was further tested by Jacobson and Trasler[10] in a study with a different strain of mice that was also highly susceptible to 6AN-induced CL. Though the susceptible and the highly similar control groups were both given 6AN, the susceptible group showed significant decreases in total nasal process area and volume, mean medial and lateral process area and volume and mean maximum head diameter as measured on serial histological sections of the embryos at the oblong or crescent stages. Overall size of the embryos was not significantly different, as measured by the crown rump lengths. The conclusions were that the hindering of normal development of the nasal processes, induced by the 6AN, might explain the predisposition of these mice to develop CL. This again supports the threshold theory of CL development, as well as the embryonic facial shape hypothesis.

Trasler and Machado[13] used two new strains of mice generated by selection, to test the CL predictive value of different craniofacial measurements. One of the new strains was susceptible to CL, the other was resistant. The length and width of the premaxilla, nasal bone length and interorbital distance were measured on newborn mice, as well as adults (75±3 days old), in both groups. A/J, C57BL/6J and CL/Fr (a known CL-susceptible strain) mice were also measured. A significant ($p \leq 0.05$) difference was found in the premaxilla length and width among the groups, with the CL-susceptible groups all being smaller for these variables. There was also a slightly smaller interorbital distance in the CL groups, and no difference in nasal bone length.

Maternal Effect of Cleft Lip

In 1969 Davidson *et al.*[18] found that there was a maternal effect on the frequency of CL in the A/J mouse. A/J males were crossed with C57BL/6J females, and then subsequently backcrossed to A/J males for six generations. Reciprocal backcrosses were obtained for each generation by backcrossing to A/J females. In each of the backcross generations, a significantly lower incidence of CL was observed with the hybrid mothers (and A/J fathers) than in the genetically similar reciprocal backcrosses with hybrid fathers (and A/J mothers). This led to the conclusion that the A/J mother either provided a different uterine environment, or perhaps lacked some factor present in the C57BL/6J strain, which increased the susceptibility of offspring to CL.

Juriloff and Fraser[21] used two strains of mice with high CL occurrence to determine whether the properties of a polygenic threshold model of inheritance would predict the frequency of CL in mice. The CL/Fr strain has over twice the frequency of CL as the A/J strain. After creating various crosses between these two strains, they found that the frequencies did not fit the model. They did find, however, that the frequency was not strongly influenced by the embryos' genotype, but was influenced by the maternal strain, and that the effect of these genes might be directed toward the survival of the embryo with CL rather than toward the actual occurrence. They also found that there was no evidence of X-linked inheritance.

Bornstein *et al.* [17] conducted a study with CL/Fr mice. They were backcrossed with C57BL/6J mice in a manner that would help determine whether there was a maternal effect on the susceptibility of CL. Not only did they find a maternal effect, but also found that the effect was not mediated through the cytoplasmic material of the maternal

gamete. They found that an embryo in a CL/Fr mother had over two times the chance of developing CL than a genetically identical embryo that developed in one of the hybrid mothers. The fact that the frequency was higher, despite identical cytoplasmic factors, negated the cytoplasmic factor mediation. They were able to deduce that the uterine environment of the CL/Fr mice may increase the CL predisposition of the embryos.

Johnston *et al.*[22] and Hansen and Hodes[31] both studied the effects of phenytoin on CL occurrence in A/J and C57BL/6J mice, as well as their F1 offspring. Although the F1 offspring were genetically similar, those with C57BL/6J mothers showed between 0%[31]-8%[22] occurrence of CL(P), whereas those with A/J mothers showed 20%[31]-67%[22] occurrence of CL(P). Not only was this maternal effect apparent in orofacial anomalies, but Hansen and Hodes also found the effects extended to resorption frequency, fetal weight and fetal length. They also found that the (B6A)F2 exhibited the same resistance as the C57BL/6J grandparents.

Trasler and Trasler[24] conducted a study comparing right, left or bilateral CL predominance in crosses among two different strains (CL/Fr and A/JFr) and one line (L line) of mice. Their results also demonstrated a maternal effect, with the A/JFr x L cross having a significantly ($p<.01$) lower incidence of cleft lip (10.3%) than the L x A/JFr cross (23.3%).

Ciriani and Diewert[19] compared the morphological differences in facial growth in yet another "A" family of mice, the A/WySn (25% CL) when crossed with the C57BL/6J. Embryos from both strains, as well as both reciprocal F1 strains, were examined at 10 days 8hours, 10 days 20 hours, 11 days 8 hours and 11 days 20 hours for stage of facial development as well as crown-rump length and somite development. They found that at

each stage of development, the A/WySn was the smallest, followed by the AWyB6F1, C57BL/6J and B6AWyF1, in order of increasing growth and development. The significant difference ($p \leq 0.05$) between the size and development of the two F1 strains indicated a maternal effect that existed in the A/WySn that retarded the growth and development of its offspring, though both F1 strains exhibited hybrid vigor over their maternal counterparts. They suggested that the maternal effect may not be directed specifically to the facial development resulting in CL, but that the CL and retarded facial development may be secondary to a generalized retardation in growth and development of the entire embryo, as compared to the other strains.

In a study done in Japan, embryos of the highly susceptible CL(P) CL/Fr mouse strain were taken at the early blastocyst stage and transferred to virgin dams of both CL/Fr mice and to the CL(P)-resistant C57BL/6J mice.[23] It was found that there was a highly significant effect on the craniofacial size of the fetuses from the two different dam strains. The craniofacial dimensions of the fetuses from the CL/Fr dams were significantly smaller than those of the C57BL/6J dams, whether they had CL(P) or not. In those fetuses that did have CL(P), the severity was significantly greater in those implanted in the CL/Fr dam strain. It was, therefore, concluded that there was an intrauterine effect on the morphogenesis of the mice.

HUMAN STUDIES

Fraser and Pashayan[1] deduced that "if the shape of the embryonic face is related to the shape of the postnatal face, and if face shape is at least in part genetically determined," and that "if face shape is indeed related to the predisposition to cleft lip," then parents of children with CL would have different facial measurements than those of

the average population. Using measurements on the subjects' face, their research found significant differences in facial shapes and morphology between two such groups. This would not only associate CL with predisposing or at least influencing genetic factors, but could also lead to information influencing predictive risks for CL by means of facial measurements.

Coccaro *et al.* [2] conducted a similar study in which they made several measurements on lateral cephalometric radiographs of 40 non-affected parents with CL(P) children and 40 non-affected parents without CL(P) children. They found significant differences in cranial base angle, upper anterior facial height, palate length and facial convexity between the two groups of parents. In the parents with CL(P) children, cranial base angle was more acute, upper anterior facial height was less, palate length was less, and the angle of facial convexity was less. There were also significant differences when fathers from both groups were compared with mothers from both groups. The fathers had a more acute cranial base angle, greater upper anterior facial height, and greater palatal length.

Kurisu *et al.*[3] also hypothesized that non-affected parents of children with CL(P) might have different morphological features than those of the general population. Their study involved 347 non-affected parents of CL(P) children, and 246 control parents. They looked at both lateral and posterior-anterior cephalograms. They too found a decrease in both upper facial height and facial convexity in the parents of CL(P) children, as compared to the control groups. They also found a tendency towards mandibular prognathism in parents of CL(P) children. Their findings were generally consistent with those of Coccaro *et al.*[2]

Nakasima and Ichinose[8] also looked for possible differences in craniofacial morphology between parents of children with CL(P) and normal controls. After making 50 different measurements on lateral and frontal cephalograms, they found that parents of CL(P) children had significantly shorter height ($p<0.01$) and greater width ($p<0.001$) in the upper face. They also found decreases in palate length, head length and both anterior and posterior upper facial height. Lower facial height was decreased in CL(P) parents, both in the anterior and posterior. They found the cranial base angle to be more obtuse in these parents, differing from the findings of Coccaro *et al.*[2]

In the preceding studies of craniofacial features of parents with CL(P), the means of the measurements of fathers and mothers were combined. This practice does not allow for the possibility that one of the parents may contribute more to the incidence of CL(P) than the other. Ward *et al.*[32] conducted an experiment using cluster analysis, in which natural groupings of parents with certain traits could be identified. Measurements were made on lateral cephalograms of parents of sporadic cases of CL(P). The cluster analysis provided an objective way of dividing the sample into natural subgroupings. It showed that 68 of the 82 cases fell into one of three major clusters, one of which showed measurements compatible with those of the normal controls, and two of which had measurement values significantly different than the controls. When an outside sampling of measurements from a group of CL(P) individuals was compared to the parent samples, it was found that they most closely resembled those of the two latter clusters. It was also noted that in 16 of the 25 parental pairs in which the parents were in two different clusters, one of the pair fell in to the "normal" cluster. These results suggest that perhaps

in some cases of isolated CL(P), predisposing factors are being contributed by only one of the parents.

Taking it a step further, Erickson[4] showed facial relationships among siblings of children with CL(P). Though small, he did find statistically significant differences in facial profile (skeletal and soft tissue)($0.10>p>0.05$), palatal form ($p<0.01$) and dental arch shape ($0.25>p>0.01$) in non-affected siblings of children with CL(P) compared to control families without history of CL(P).

MODES OF INHERITANCE

The studies of the mouse facial dimensions have largely addressed the question of predisposing facial shape and maternal effect on CL(P). A study by Bingle and Niswander[33] indicated that a major maternal effect does not exist for human CL(P). Human studies confirm, however, that there are measurable differences between both parents and siblings of children with CL(P), though it has not been determined exactly how these traits are inherited. Though the occurrence of CL(P) exhibits some threshold characteristics, the pattern of inheritance also suggests the possibility of genomic imprinting. Sapienza[34] explains that

> "...imprinting implies that the phenotype observed for a particular gene or collection of genes varies, depending on the sex of the parent from which the gamete containing that gene or genes originated...If the phenotype yielded by a particular gene is different when that gene is maternally inherited versus paternally inherited, then that gene is said to be imprinted."

The purpose of this study is to further determine the relationship of facial shape and clefting in specific parent strains in mice with that of their offspring. This will aid in the search for possible genetic determinants for CL(P), which will greatly enhance the diagnostic and treatment capabilities of the orthodontists who treat these patients.

SPECIFIC AIM

The specific aim of this project is to compare the craniofacial shapes of two different F1 hybrid strains of mice that are genetically the same as each other, as well as with each of their two parental strains, A/J and C57BL/6J (C57). One of the parental strains, A/J, has a predisposition for CL(P) and the other (C57), has no occurrence of CL(P).

HYPOTHESIS

The null hypothesis for this research experiment is that there will be no differences in craniofacial shape between the AB6F1/J (AB6) and B6AF1/J (B6A), since both groups will be genetically identical. The shape the F1 strains will fall somewhere between the shapes of the parental strains, A/J and C57.

An alternate hypothesis is that there will be differences in craniofacial shape between the two F1 strains. The shape of the craniofacial structures will be closely related to the maternal strain.

MATERIALS AND METHODS

SPECIMEN PREPARATION

All mice used in this study had been previously obtained from the Jackson Laboratory (Bar Harbor, ME), and skulls were available in the Department of Oral Facial Development. The mice had been euthanized between 36-44 days of age, and all soft tissue had been removed. The preparation of the skulls resulted in both hemi-mandibles being disarticulated from the cranium. The dried skulls had then been varnished with a clear polyurethane spray. Dried, varnished skulls from A/J, C57, AB6, and B6A mice were used in this study. At least 6 male and 6 female specimens were obtained from each group of mice. Sample size was a sample of convenience, and similar sample sizes have been used in references cited.[10, 13, 23, 25]

PHOTOGRAPHY

Each dried skull was digitally photographed from the basilar aspect at a resolution of 12 megapixels, using a Nikon DXM1200 digital camera (Melville, NY) attached to a Leica GZ6 stereomicroscope (Bannockburn, IL). A piece of rectangular orthodontic wire of known dimension (1.56 mm x 0.55 mm) was placed with each specimen so that precise measurements could be obtained at any magnification. The wire was oriented in the same plane as the landmarks. The specimens were numbered and randomized so that the investigator was blind as to the strain of mouse being analyzed. Images were saved as J-peg files on CD-R media.

DIGITIZING IMAGES

The images were uploaded into image digitizing software (Didge 2.2, Parthenogenetic Products, Omaha, NE), and various landmarks were identified by the examiner on each specimen. The software assigned X- and Y-coordinates to each landmark. These coordinates were then transferred to a Microsoft Excel spreadsheet. The landmarks are described in Table I, and illustrated in Figure 1.

MEASUREMENTS

Formulas were used in the spreadsheet to calculate the measurements between certain predetermined landmarks. Seven measurements are described in Table II, and can be seen in Figure 2. Bilateral measurements (zygomatic arch length and palatine fissure length) were averaged together to form one number for each specimen. All specimens were measured twice, then a third time if there was >10% difference between the two measurements. Data from the two measurements were averaged, and the means were used in the statistical analyses. If a third measurement was made due to excessive error, the two measurements with the least difference were averaged together.

To account for the innate size difference present in each specimen, ratios were created using pairs of measurements that were matched together to form a proportion or index. This allowed facial shapes to be analyzed, as opposed to pure linear measurements. The ratios analyzed included:

A) interfissure length : premaxilla width (measurements 1:4)

B) premaxilla width : palatine fissure length (measurements 4:2)

C) premaxilla width : zygomatic arch length (measurements 4:3)

D) premaxilla width : intraorbital width (measurements 4:5)

E) intermolar width : premaxilla width (measurements 6:4)

F) palatine bone width : premaxilla width (measurements 7:4)

STATISTICAL ANALYSIS

The data collected were then analyzed using the Statistical Package for the Social Sciences (SPSS®) Version 11.5.0 software for Windows® (Chicago, IL). T-tests were performed to determine if there were significant differences between the males and females for each measurement. A oneway analysis of variance (ANOVA) model was used to examine the differences in the means of each measurement between the two parent strains, between the two F1 strains, and between the two F1 strains and the two parent strains. Univariate ANOVA of the measurements were performed using weight as a covariate in an attempt to eliminate size as a factor in the analyses. Statistical significance was tested to a $p \leq 0.05$. Finally, discriminant analyses were performed to determine whether the measurements or ratios of each specimen could be used collectively as a multivariate character to define the shape of the face.

RESULTS

MEASUREMENT ERROR

Only one of the 441 measurements exhibited an error of >10% between the first two measurements gathered. A third measurement was within 10% of the second, so the two latter were averaged for the measurement. The mean measurement error for all measurements was 2.38%.

DESCRIPTIVE STATISTICS

Descriptive statistics run on all strains for all the measurements gathered showed no obvious outliers for any of the seven measurements (Table III). T-tests performed on each measurement and ratio between the males and females showed no significant differences in any of the measurements (Table IV, V). Since there were no significant differences between the sexes, it was determined to combine the two in making further comparisons of shapes and measurements.

COMPARISON OF MEANS

Measurements

Graphs of the means of each measurement can be seen in Figures 3-9. The one-way ANOVA comparing the means of each of the measurements among the four strains showed that the two parent strains differed significantly ($P<0.05$) from each other in 6 of the 7 measurements, with palatine fissure length being the only measurement which

showed no significant difference (Table VI). The two F1 strains differed significantly from each other in only 4 of the 7 measurements.

All measurements of the AB6 strain differed significantly from the A/J parental strain, and zygomatic arch length was the only measurement in which AB6 was not significantly different from the C57 parental strain. The B6A differed significantly from the A/J parental strain in 4 measurements, whereas it differed from the C57 parental strain in only 1 measurement.

Results of the univariate ANOVA controlling for weight produced slightly different results (Table VII). Although there were still 6 of 7 measurements that differed significantly between the parental strains, it was the zygomatic arch length which did not differ as opposed to the palatine fissure length. The two F1 strains differed significantly from each other in only 1 of the 7 measurements (palatine bone width).

The F1 strains showed similar differences when weight was used as a covariate as when it was not. The AB6, however, only differed significantly from the A/J in 5 of the measurements instead of all 7.

Ratios

Since the ratios of the facial measurements help to factor out size as a variant, analyses of the means were not performed on these data using weight-controlled ANOVA. The results of the oneway ANOVA for the ratios can be seen in Table VIII. The ratio of palatine bone width to premaxilla width showed no significant difference between any of the four strains. All of the other ratios showed significant difference between the parent strains. Half of the ratios were different between the AB6 and each

parent strain. Half of the ratios were also different between the B6A and the C57, but there were only 2 ratios that showed significant differences between the B6A and the A/J.

DISCRIMINANT ANALYSIS

Measurements

Discriminant analysis uses all of the measurements on each individual specimen collectively as a single multivariate character to help define a face shape. The statistics used to determine the different functions of the analysis can be seen in Table IX, and the actual structure matrices can be seen in Table X. Once the functions are determined, each individual is categorized by the analysis into the group which best fits the calculated functions. A cross-validation is performed in which each case is classified by the functions derived from all cases other than that case. Figure 10 illustrates the grouping attained by the discriminant functions.

Of the three functions generated, most of the discriminating ability lies within the first function (87.2%). A chi-square value of 18.76 for function 3 indicates that a significant amount of discriminating ability still exists. The analysis correctly predicted group membership for 100% of the A/J and C57 parent strains, 93.3% of the AB6 strain and 83.3% of the B6A strain (Table XI). The one incorrectly classified specimen of the AB6 strain was placed in the B6A category. The two incorrectly classified specimens of the B6A strain were placed in the AB6 category. Cross-validation of the data produced similar results, except that two of the A/J strain (16.7%) were incorrectly classified into the B6A category.

Ratios

The statistics used to determine the different functions of the discriminant analysis of the ratios can be seen in Table XII, and the actual structure matrices can be seen in Table XIII. Figure 11 illustrates the grouping attained by the discriminant functions.

The first function of the analysis for the ratios exhibited 92.4% o the discriminating ability. The predicting value of this analysis was similar to the one done with the measurements (Table XIV). It correctly classified 100% of the two parental strains, 93.3% of the AB6 strain and 75.0% of the B6A strain. The misclassifications were to the same categories as previously described, with the one AB6 specimen placed in the B6A category, and the 3 B6A specimens placed in the AB6 category. Upon cross validation, one A/J was misclassified as a B6A, one AB6 was misclassified as a B6A, and three B6A were misclassified as AB6.

FIGURES AND TABLES

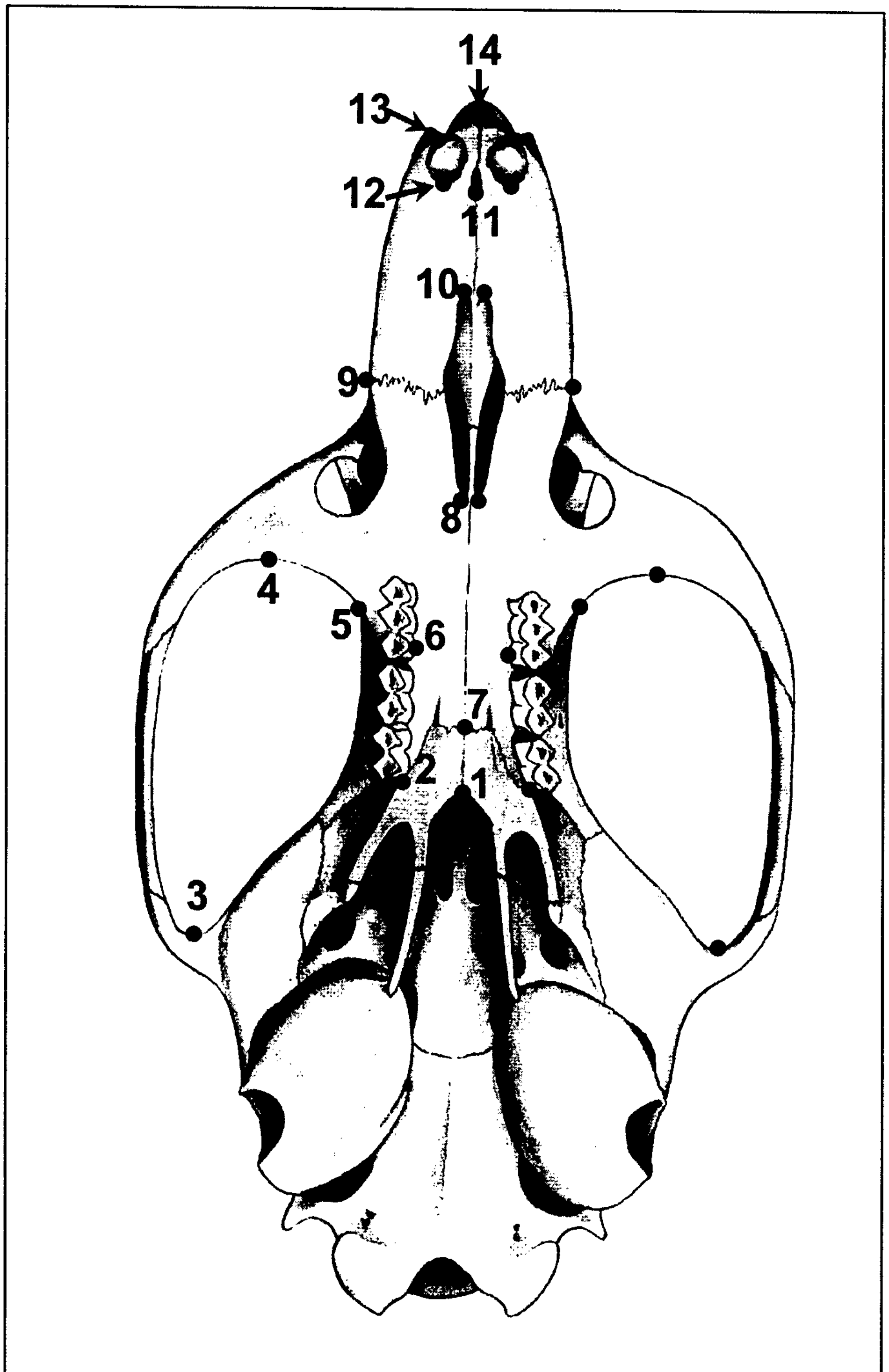

FIGURE 1. Ventral diagram of mouse cranium. Dots indicate points digitized in software.

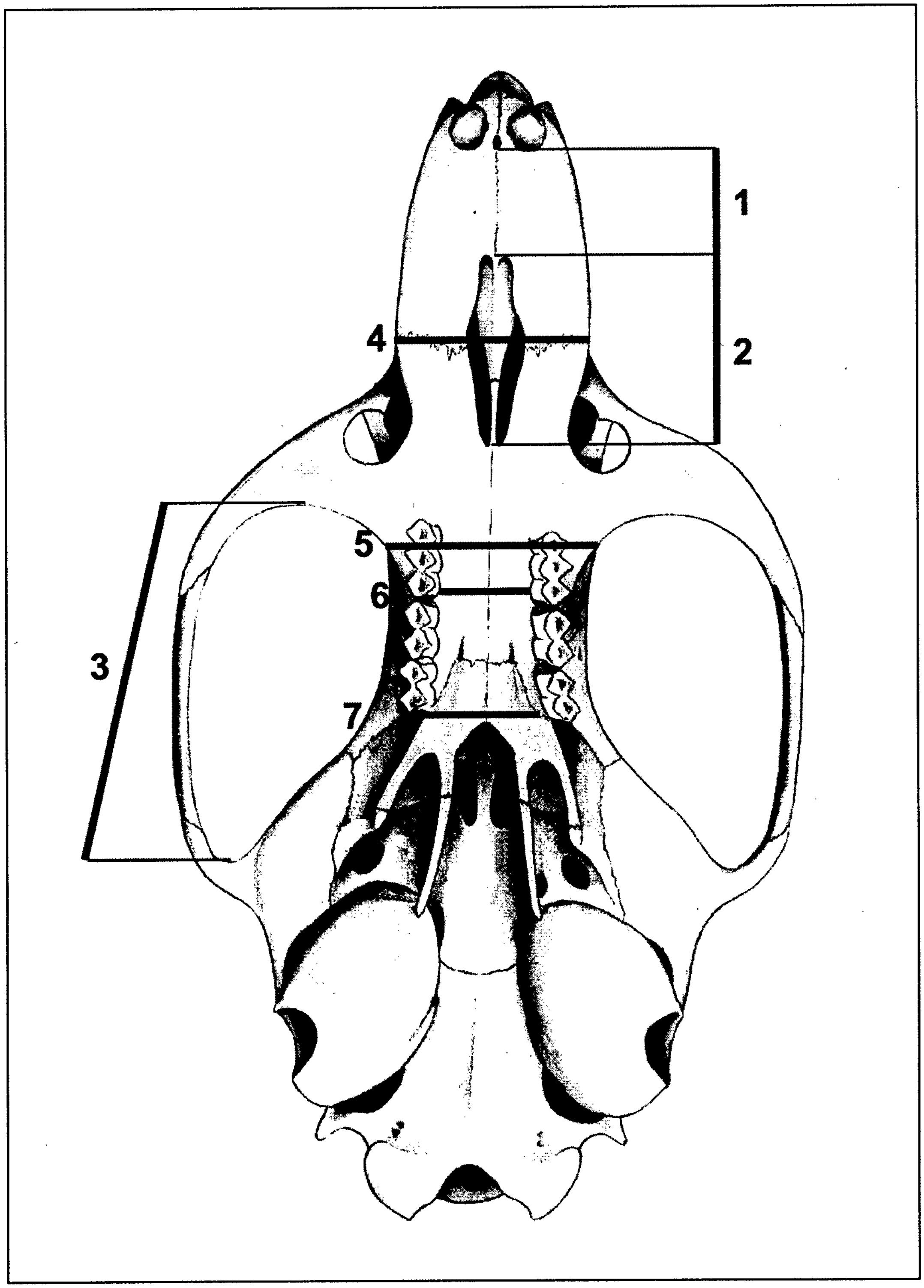

FIGURE 2. Identification of measurements used for analyses.

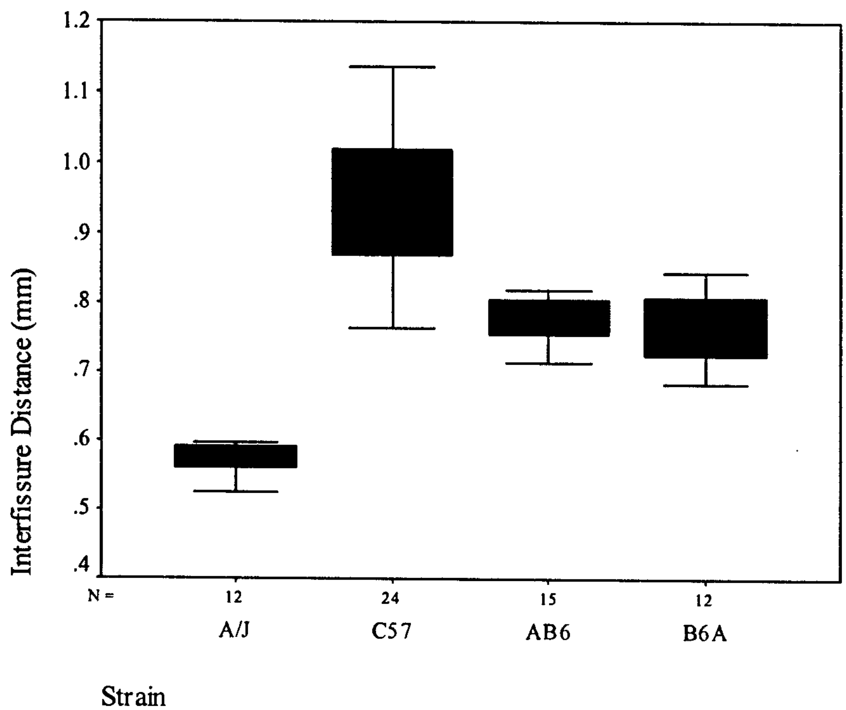

FIGURE 3. Boxplot of interfissure distance for the four strains of mice. Bars illustrate means, and boxes illustrate interquartile range.

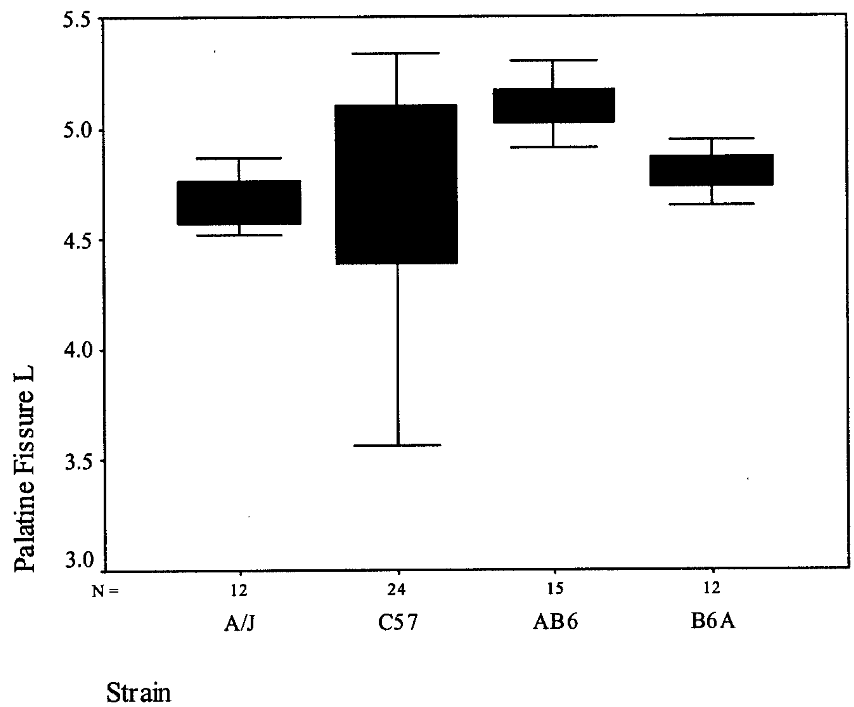

FIGURE 4. Boxplot of palatine fissure length for the four strains of mice. Bars illustrate means, and boxes illustrate interquartile range.

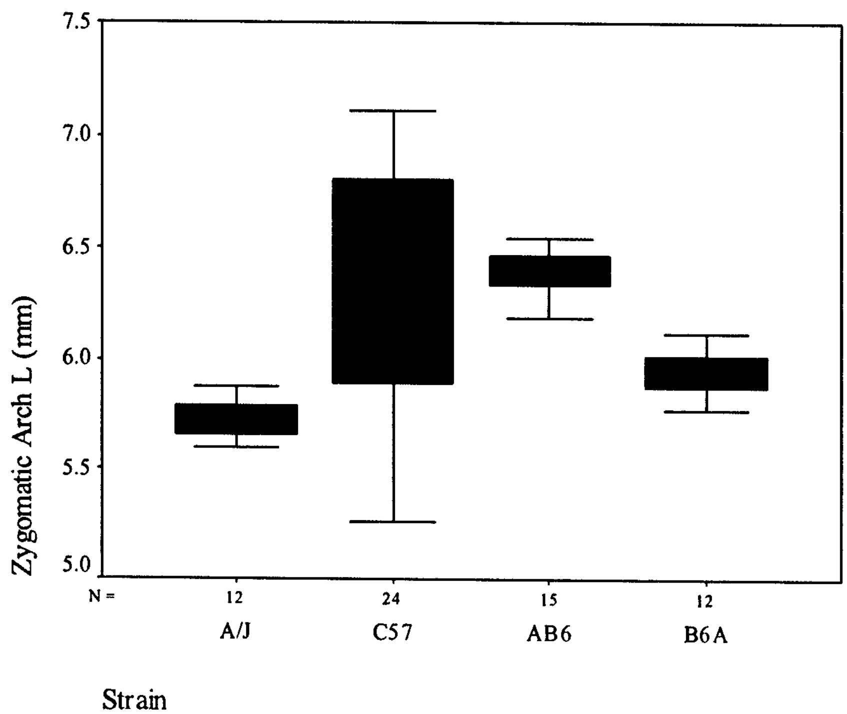

FIGURE 5. Boxplot of the zygomatic arch length for the four strains of mice. Bars illustrate means, and boxes illustrate interquartile range.

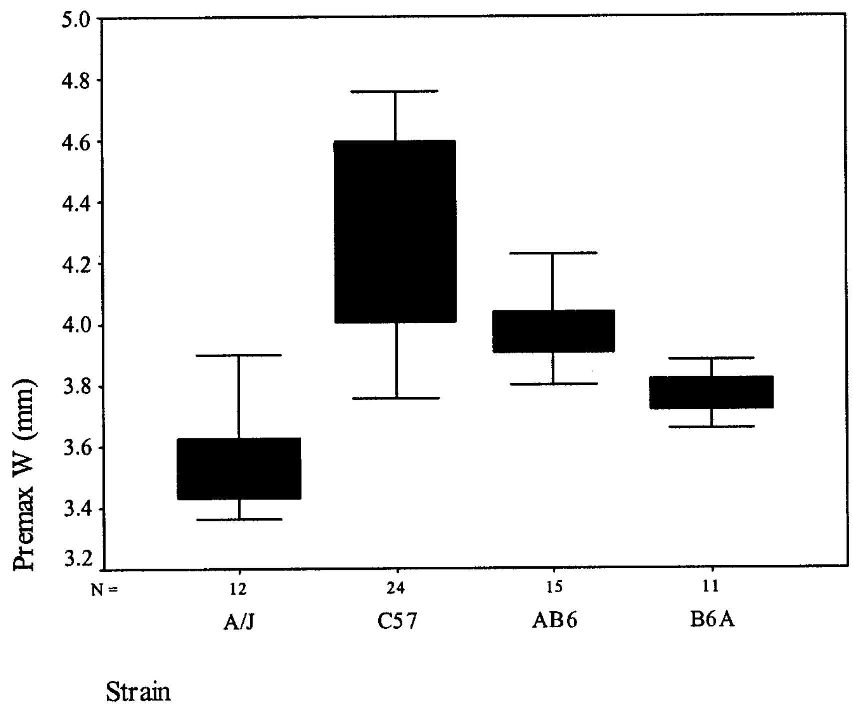

FIGURE 6. Boxplot of premaxillary width for the four strains of mice. Bars illustrate means, and boxes illustrate interquartile range.

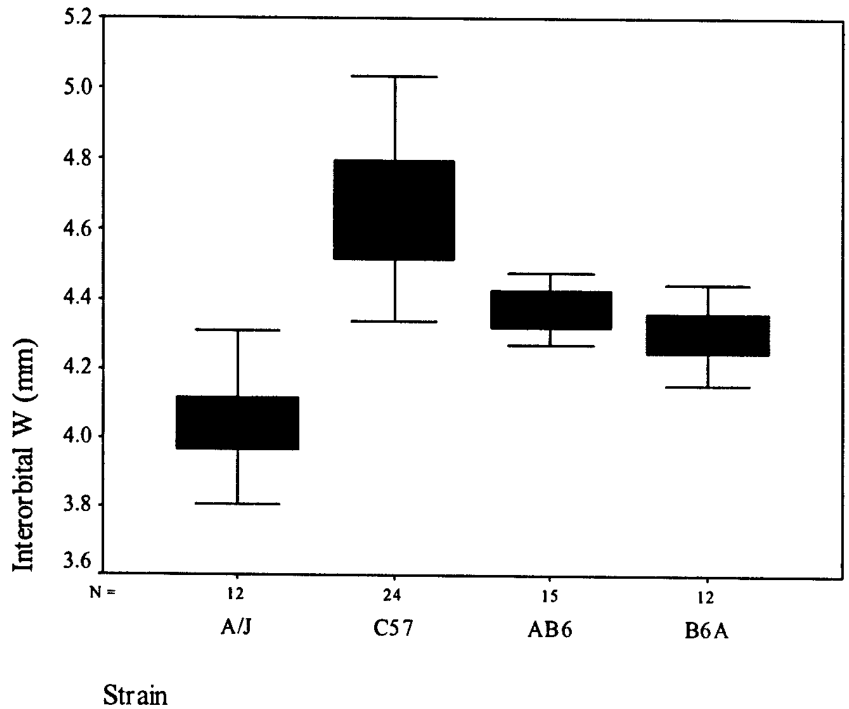

FIGURE 7. Boxplot of interorbital width for the four strains of mice. Bars illustrate means, and boxes illustrate interquartile range.

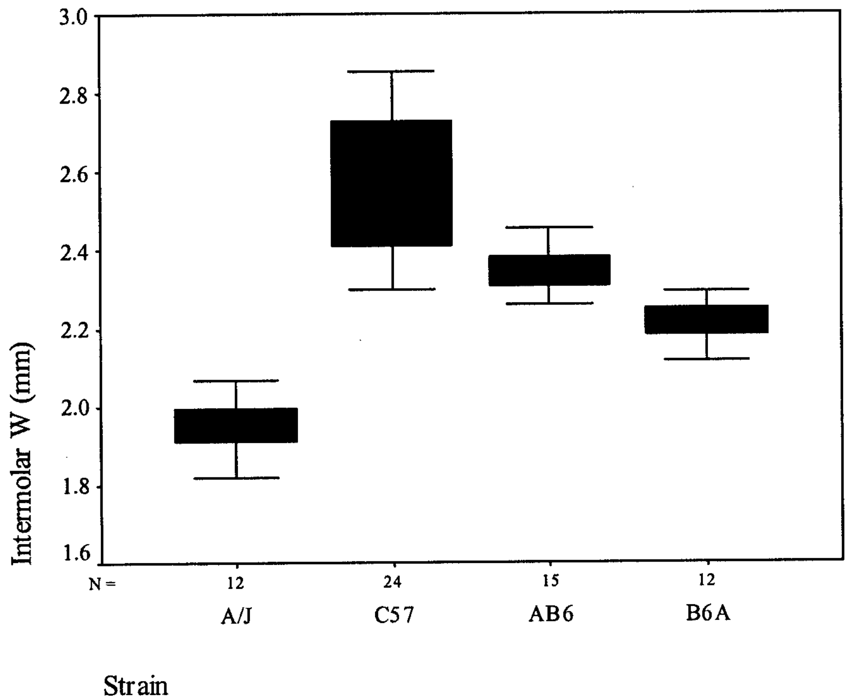

FIGURE 8. Boxplot of intermolar width for the four strains of mice. Bars illustrate means, and boxes illustrate interquartile range.

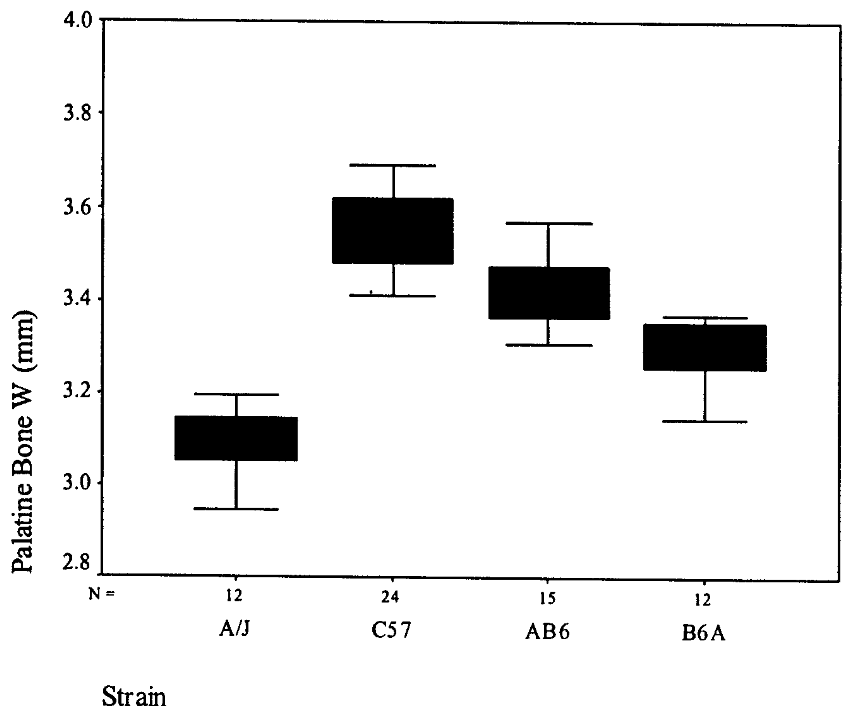

FIGURE 9. Boxplot of palatine bone width for the four strains of mice. Bars illustrate means, and boxes illustrate interquartile range.

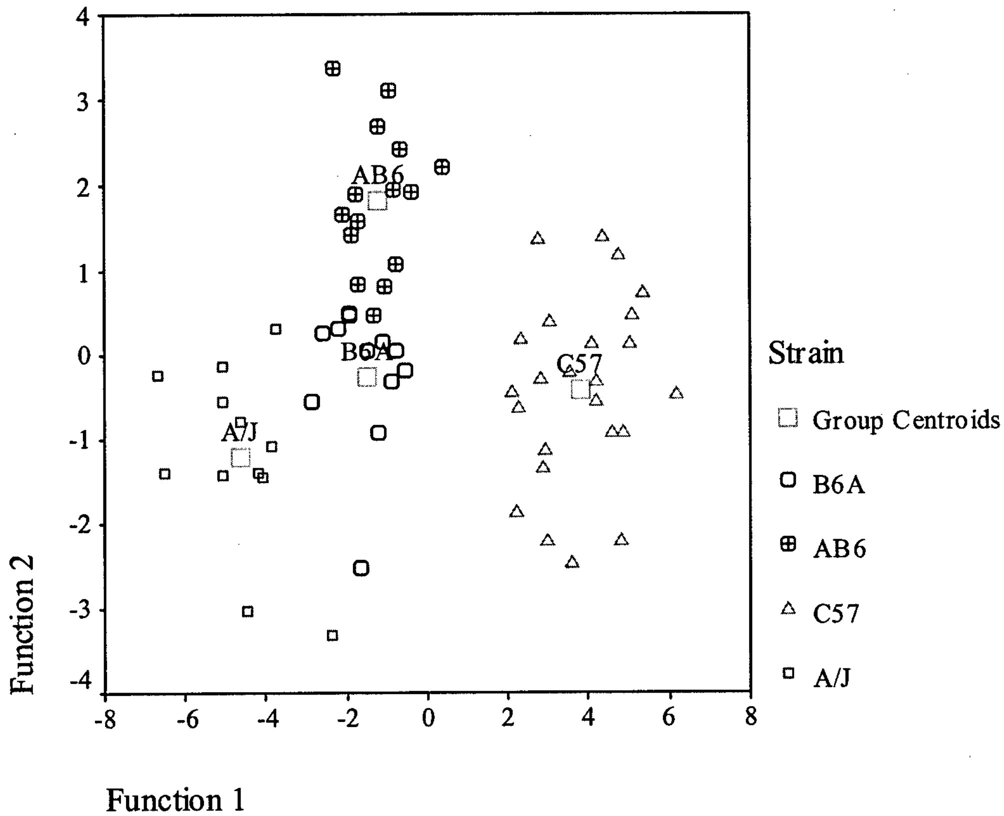

FIGURE 10. Plot of discriminant functions of the measurements.

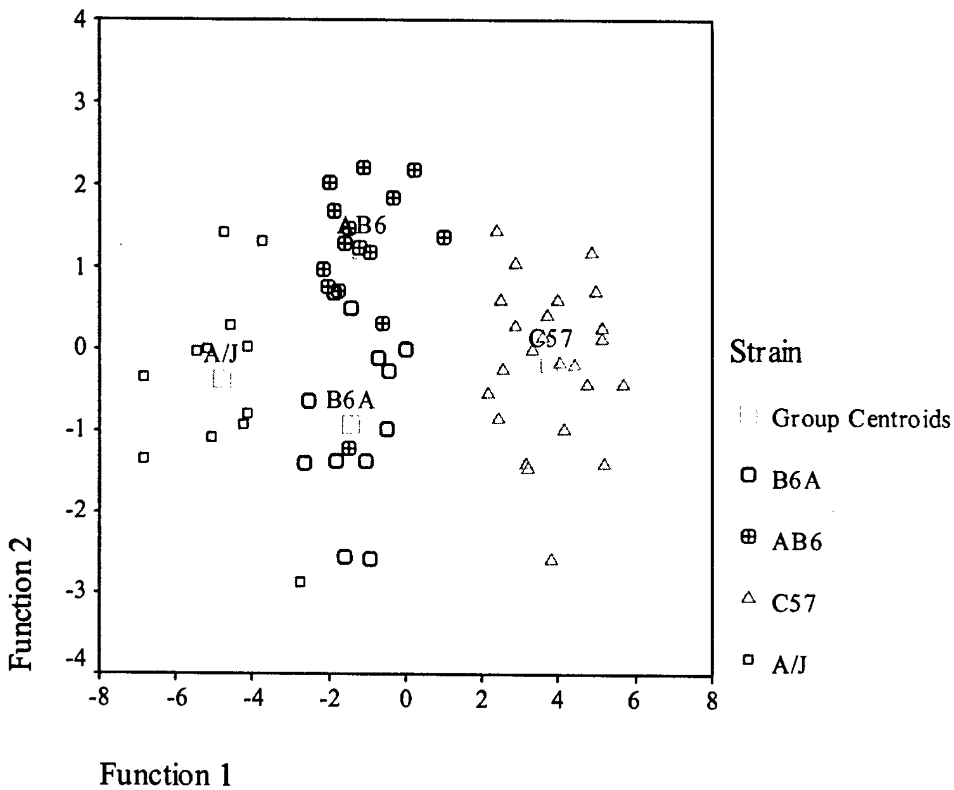

FIGURE 11. Plot of discriminant functions of the ratios.

TABLE I

Desscription of anatomical landmarks identified on skulls

Midline Landmark	Description
1	Posterior midline suture of palatine bone
7	Anterior midline suture of palatine bone
11	Posterior aspect of incisive foramen
14	Anterior most aspect of incisive bones
Bilateral Lanmarks	
2	Lateral margin of the palatine-external pterygoid suture
3	Posterior apex of zygomatic arch
4	Anterior apex of zygomatic arch
5	Junction of zygomatic process of maxilla and frontal bone
6	Medial most aspect of maxillary first molar alveoli
8	Posterior apex of palatine fissure
9	Lateral most aspect of incisive-maxillary suture
10	Anterior apex of palatine fissure
12	Posterior apex of maxillary incisor alveolus
13	Anterolateral aspect of maxillary incisor

TABLE II

Descriptions of the cranial measurements calculated for each specimen

Measurement	Name	Description
1	Interfissure length	Length between incisive foramen and palatine fissure
2	Palatine fissure length	Longest length of right and left palatine fissures
3	Zygomatic arch length	Greatest length anterior to posterior of the zygomatic arch
4	Premaxilla width	Distance between lateral aspects of incisive-maxillary suture
5	Interorbital width	Distance between frontal bones at the junction of the zygomatic arch
6	Intermolar width	Distance between distal roots of maxillary first molars
7	Palatine bone width	Palatine bone width at the lateral margins of the palatine-external pterygoid sutures

TABLE III

Descriptive statistics for each measurement made on the four strains of mice

	N	Minimum	Maximum	Mean	Std. Deviation
Weight	63	13.07	37.70	20.808	5.929
Interfissure Distance (1)	63	.52	1.14	.807	.155
Palatine Fissure L (2)	63	3.56	5.34	4.814	.325
Zygomatic Arch L (3)	63	5.26	7.11	6.146	.424
Premax W (4)	62	3.36	4.76	3.982	.363
Interorbital W (5)	63	3.80	5.04	4.409	.265
Intermolar W (6)	63	1.82	2.86	2.338	.262
Palatine Bone W (7)	63	2.95	3.91	3.398	.203
Valid N (listwise)	62				

TABLE IV

Descriptive statistics for measurements and ratios by gender

	Gender	N	Mean	Std. Deviation	Std. Error Mean
Weight	M	30	21.879	5.486	1.002
	F	33	19.834	6.228	1.084
Interfissure Distance (1)	M	30	0.796	0.131	0.024
	F	33	0.817	0.175	0.030
Palatine Fissure L (2)	M	30	4.882	0.267	0.049
	F	33	4.752	0.362	0.063
Zygomatic Arch L (3)	M	30	6.160	0.332	0.061
	F	33	6.133	0.498	0.087
Premax W (4)	M	29	3.965	0.295	0.055
	F	33	3.998	0.418	0.073
Interorbital W (5)	M	30	4.359	0.215	0.039
	F	33	4.454	0.299	0.052
Intermolar W (6)	M	30	2.296	0.228	0.042
	F	33	2.375	0.289	0.050
Palatine Bone W (7)	M	30	3.386	0.184	0.034
	F	33	3.410	0.221	0.039
IFL/Premax W (A)	M	29	0.200	0.024	0.005
	F	33	0.203	0.027	0.005
Premax W/Palatine Fissure L (B)	M	29	0.814	0.065	0.012
	F	33	0.843	0.084	0.015
Premax W/Zyg Arch L (C)	M	29	0.643	0.031	0.006
	F	33	0.652	0.035	0.006
Premax W/Intraorbital W (D)	M	29	0.908	0.032	0.006
	F	33	0.895	0.041	0.007
Intermolar W/Premax W (E)	M	29	0.580	0.028	0.005
	F	33	0.593	0.022	0.004
Palatine Bone W/Premax W (F)	M	29	0.857	0.043	0.008
	F	33	0.858	0.056	0.010

TABLE V

T-tests for equality of means between genders for each measurement and ratio

	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
Weight	1.377	61	0.173	2.045	1.485
Measurements:					
Interfissure Distance (1)	-0.530	61	0.598	-0.021	0.039
Palatine Fissure L (2)	1.612	61	0.112	0.130	0.081
Zygomatic Arch L (3)	0.251	61	0.803	0.027	0.108
Premax W (4)	-0.354	60	0.724	-0.033	0.093
Interorbital W (5)	-1.434	61	0.157	-0.095	0.066
Intermolar W (6)	-1.197	61	0.236	-0.079	0.066
Palatine Bone W (7)	-0.461	61	0.647	-0.024	0.052
Ratios:					
IFL/Premax W (A)	-0.469	60	0.641	-0.003	0.007
Premax W/Palatine Fissure L (B)	-1.514	60	0.135	-0.029	0.019
Premax W/Zyg Arch L (C)	-1.030	60	0.307	-0.009	0.008
Premax W/Intraorbital W (D)	1.374	60	0.175	0.013	0.009
Intermolar W/Premax W (E)	-1.965	60	0.054	-0.013	0.006
Palatine Bone W/Premax W (F)	-0.104	60	0.918	-0.001	0.013

TABLE VI

Oneway ANOVA of the measurement means

Dependent Variable	(I) Strain	(J) Strain	Mean Difference (I-J)	Std. Error	Sig.
Interfissure Distance (1)	A/J	C57	-0.3564 *	0.0303	0.000
		AB6	-0.2011 *	0.0332	0.000
		B6A	-0.1804 *	0.0350	0.000
	C57	A/J	0.3564 *	0.0303	0.000
		AB6	0.1553 *	0.0282	0.000
		B6A	0.1760 *	0.0303	0.000
	AB6	A/J	0.2011 *	0.0332	0.000
		C57	-0.1553 *	0.0282	0.000
		B6A	0.0207	0.0332	1.000
	B6A	A/J	0.1804 *	0.0350	0.000
		C57	-0.1760 *	0.0303	0.000
		AB6	-0.0207	0.0332	1.000
Palatine Fissure L (2)	A/J	C57	-0.0671	0.1019	1.000
		AB6	-0.4333 *	0.1116	0.002
		B6A	-0.1246	0.1176	1.000
	C57	A/J	0.0671	0.1019	1.000
		AB6	-0.3662 *	0.0948	0.002
		B6A	-0.0576	0.1019	1.000
	AB6	A/J	0.4333 *	0.1116	0.002
		C57	0.3662 *	0.0948	0.002
		4	0.3087 *	0.1116	0.045
	B6A	A/J	0.1246	0.1176	1.000
		C57	0.0576	0.1019	1.000
		AB6	-0.3087 *	0.1116	0.045

* The mean difference is significant at the .05 level.

(continued)

TABLE VI

Oneway ANOVA of the measurement means

(continued)

Dependent Variable	(I) Strain	(J) Strain	Mean Difference (I-J)		Std. Error	Sig.
Zygomatic Arch L (3)	A/J	C57	-0.6221	*	0.1198	0.000
		AB6	-0.6612	*	0.1312	0.000
		B6A	-0.2328		0.1383	0.586
	C57	A/J	0.6221	*	0.1198	0.000
		AB6	-0.0391		0.1115	1.000
		B6A	0.3892	*	0.1198	0.011
	AB6	A/J	0.6612	*	0.1312	0.000
		C57	0.0391		0.1115	1.000
		4	0.4284	*	0.1312	0.011
	B6A	A/J	0.2328		0.1383	0.586
		C57	-0.3892	*	0.1198	0.011
		AB6	-0.4284	*	0.1312	0.011
Premax W	A/J	C57	-0.7582	*	0.0776	0.000
		AB6	-0.4504	*	0.0850	0.000
		B6A	-0.2296		0.0917	0.090
	C57	A/J	0.7582	*	0.0776	0.000
		AB6	0.3078	*	0.0723	0.000
		B6A	0.5286	*	0.0800	0.000
	AB6	A/J	0.4504	*	0.0850	0.000
		C57	-0.3078	*	0.0723	0.000
		B6A	0.2208		0.0872	0.084
	B6A	A/J	0.2296		0.0917	0.090
		C57	-0.5286	*	0.0800	0.000
		AB6	-0.2208		0.0872	0.084

* The mean difference is significant at the .05 level.

(continued)

TABLE VI

Oneway ANOVA of the measurement means

(continued)

Dependent Variable	(I) Strain	(J) Strain	Mean Difference (I-J)	Std. Error	Sig.
Interorbital W (5)	A/J	C57	-0.6216 *	0.0472	0.000
		AB6	-0.3357 *	0.0517	0.000
		B6A	-0.2722 *	0.0545	0.000
	C57	A/J	0.6216 *	0.0472	0.000
		AB6	0.2859 *	0.0439	0.000
		B6A	0.3494 *	0.0472	0.000
	AB6	A/J	0.3357 *	0.0517	0.000
		C57	-0.2859 *	0.0439	0.000
		B6A	0.0635	0.0517	1.000
	B6A	A/J	0.2722 *	0.0545	0.000
		C57	-0.3494 *	0.0472	0.000
		AB6	-0.0635	0.0517	1.000
Intermolar W (6)	A/J	C57	-0.6331 *	0.0425	0.000
		AB6	-0.3991 *	0.0465	0.000
		B6A	-0.2691 *	0.0491	0.000
	C57	A/J	0.6331 *	0.0425	0.000
		AB6	0.2339 *	0.0395	0.000
		B6A	0.3640 *	0.0425	0.000
	AB6	A/J	0.399A/J *	0.0465	0.000
		C57	-0.2339 *	0.0395	0.000
		B6A	0.1301 *	0.0465	0.042
	B6A	A/J	0.2691 *	0.0491	0.000
		C57	-0.3640 *	0.0425	0.000
		AB6	-0.1301 *	0.0465	0.042

* The mean difference is significant at the .05 level.

(continued)

TABLE VI

Oneway ANOVA of the measurement means

(continued)

Dependent Variable	(I) Strain	(J) Strain	Mean Difference (I-J)	Std. Error	Sig.
Palatine Bone W (7)	A/J	C57	-0.4719 *	0.0365	0.000
		AB6	-0.3150 *	0.0400	0.000
		B6A	-0.1919 *	0.0422	0.000
	C57	A/J	0.4719 *	0.0365	0.000
		AB6	0.1568 *	0.0340	0.000
		B6A	0.2799 *	0.0365	0.000
	AB6	A/J	0.3150 *	0.0400	0.000
		C57	-0.1568 *	0.0340	0.000
		B6A	0.1231 *	0.0400	0.019
	B6A	A/J	0.1919 *	0.0422	0.000
		C57	-0.2799 *	0.0365	0.000
		AB6	-0.1231 *	0.0400	0.019

* The mean difference is significant at the .05 level.

TABLE VII

Univariate ANOVA of the measurements
controlling for weight

Dependent Variable	(I) Strain	(J) Strain	Mean Difference (I-J)	Std. Error	Sig.[a]
Interfissure Distance (1)	A/J	C57	-0.265 *	0.028	0.000
		AB6	-0.111 *	0.030	0.003
		B6A	-0.151 *	0.028	0.000
	C57	A/J	0.265 *	0.028	0.000
		AB6	0.155 *	0.022	0.000
		B6A	0.114 *	0.026	0.000
	AB6	A/J	0.111 *	0.030	0.003
		C57	-0.155 *	0.022	0.000
		B6A	-0.041	0.028	0.904
	B6A	A/J	0.151 *	0.028	0.000
		C57	-0.114 *	0.026	0.000
		AB6	0.041	0.028	0.904
Palatine Fissure L (2)	A/J	C57	0.337 *	0.067	0.000
		AB6	-0.033	0.072	1.000
		B6A	0.004	0.067	1.000
	C57	A/J	-0.337 *	0.067	0.000
		AB6	-0.370 *	0.053	0.000
		B6A	-0.333 *	0.062	0.000
	AB6	A/J	0.033	0.072	1.000
		C57	0.370 *	0.053	0.000
		B6A	0.037	0.067	1.000
	B6A	A/J	-0.004	0.067	1.000
		C57	0.333 *	0.062	0.000
		AB6	-0.037	0.067	1.000

Based on estimated marginal means

* The mean difference is significant at the .05 level.

[a] Adjustment for multiple comparisons: Bonferroni.

(continued)

TABLE VII

Univariate ANOVA of the measurements controlling for weight

(continued)

Dependent Variable	(I) Strain	(J) Strain	Mean Difference (I-J)	Std. Error	Sig.[a]
Zygomatic arch L (3)	A/J	C57	-0.150	0.080	0.397
		AB6	-0.194	0.086	0.165
		B6A	-0.082	0.080	1.000
	C57	A/J	0.150	0.080	0.397
		AB6	-0.043	0.063	1.000
		B6A	0.068	0.074	1.000
	AB6	A/J	0.194	0.086	0.165
		C57	0.043	0.063	1.000
		B6A	0.111	0.080	1.000
	B6A	A/J	0.082	0.080	1.000
		C57	-0.068	0.074	1.000
		AB6	-0.111	0.080	1.000
Premax W (4)	A/J	C57	-0.445 *	0.049	0.000
		AB6	-0.140	0.052	0.054
		B6A	-0.138 *	0.049	0.041
	C57	A/J	0.445 *	0.049	0.000
		AB6	0.305 *	0.038	0.000
		B6A	0.307 *	0.046	0.000
	AB6	A/J	0.140	0.052	0.054
		C57	-0.305 *	0.038	0.000
		B6A	0.002	0.050	1.000
	B6A	A/J	0.138 *	0.049	0.041
		C57	-0.307 *	0.046	0.000
		AB6	-0.002	0.050	1.000

Based on estimated marginal means

* The mean difference is significant at the .05 level.

[a] Adjustment for multiple comparisons: Bonferroni.

(continued)

TABLE VII

Univariate ANOVA of the measurements controlling for weight

(continued)

Dependent Variable	(I) Strain	(J) Strain	Mean Difference (I-J)	Std. Error	Sig.[a]
Interorbital W (5)	A/J	C57	-0.492 *	0.046	0.000
		AB6	-0.207 *	0.049	0.000
		B6A	-0.231 *	0.046	0.000
	C57	A/J	0.492 *	0.046	0.000
		AB6	0.285 *	0.036	0.000
		B6A	0.261 *	0.042	0.000
	AB6	A/J	0.207 *	0.049	0.000
		C57	-0.285 *	0.036	0.000
		B6A	-0.024	0.046	1.000
	B6A	A/J	0.231 *	0.046	0.000
		C57	-0.261 *	0.042	0.000
		AB6	0.024	0.046	1.000
Intermolar W (6)	A/J	C57	-0.480 *	0.033	0.000
		AB6	-0.248 *	0.035	0.000
		B6A	-0.220 *	0.033	0.000
	C57	A/J	0.480 *	0.033	0.000
		AB6	0.233 *	0.026	0.000
		B6A	0.260 *	0.030	0.000
	AB6	A/J	0.248 *	0.035	0.000
		C57	-0.233 *	0.026	0.000
		B6A	0.027	0.033	1.000
	B6A	A/J	0.220 *	0.033	0.000
		C57	-0.260 *	0.030	0.000
		AB6	-0.027	0.033	1.000

Based on estimated marginal means

* The mean difference is significant at the .05 level.

[a] Adjustment for multiple comparisons: Bonferroni.

(continued)

TABLE VII

Univariate ANOVA of the measurements controlling for weight

(continued)

Dependent Variable	(I) Strain	(J) Strain	Mean Difference (I-J)		Std. Error	Sig.[a]
Palatine Bone W (7)	A/J	C57	-0.488	*	0.043	0.000
		AB6	-0.331	*	0.046	0.000
		B6A	-0.197	*	0.043	0.000
	C57	A/J	0.488	*	0.043	0.000
		AB6	0.157	*	0.034	0.000
		B6A	0.291	*	0.040	0.000
	AB6	A/J	0.331	*	0.046	0.000
		C57	-0.157	*	0.034	0.000
		B6A	0.134	*	0.043	0.017
	B6A	A/J	0.197	*	0.043	0.000
		C57	-0.291	*	0.040	0.000
		AB6	-0.134	*	0.043	0.017

Based on estimated marginal means

* The mean difference is significant at the .05 level.

[a] Adjustment for multiple comparisons: Bonferroni.

TABLE VIII

Oneway ANOVA of the ratio means

Dependent Variable	(I) Strain	(J) Strain	Mean Difference (I-J)	Std. Error	Sig.
IFL/Premax W (A)	A/J	C57	-0.054 *	0.006	0.000
		AB6	-0.032 *	0.007	0.000
		B6A	-0.039 *	0.007	0.000
	C57	A/J	0.054 *	0.006	0.000
		AB6	0.022 *	0.006	0.002
		B6A	0.015	0.006	0.104
	AB6	A/J	0.032 *	0.007	0.000
		C57	-0.022 *	0.006	0.002
		B6A	-0.007	0.007	1.000
	B6A	A/J	0.039 *	0.007	0.000
		C57	-0.015	0.006	0.104
		AB6	0.007	0.007	1.000
Premax W/Palatine Fissure L (B)	A/J	C57	-0.152 *	0.014	0.000
		AB6	-0.023	0.015	0.739
		B6A	-0.029	0.016	0.453
	C57	A/J	0.152 *	0.014	0.000
		AB6	0.129 *	0.013	0.000
		B6A	0.123 *	0.014	0.000
	AB6	A/J	0.023	0.015	0.739
		C57	-0.129 *	0.013	0.000
		B6A	-0.006	0.015	1.000
	B6A	A/J	0.029	0.016	0.453
		C57	-0.123 *	0.014	0.000
		AB6	0.006	0.015	1.000

1 The mean difference is significant at the .05 level.

(continued)

TABLE VIII

Oneway ANOVA of the ratio means

(continued)

Dependent Variable	(I) Strain	(J) Strain	Mean Difference (I-J)	Std. Error	Sig.
Premax W/Zyg Arch L ©	A/J	C57	-0.061 *	0.007	0.000
		AB6	-0.007	0.008	1.000
		B6A	-0.018	0.008	0.218
	C57	A/J	0.061 *	0.007	0.000
		AB6	0.054 *	0.007	0.000
		B6A	0.043 *	0.007	0.000
	AB6	A/J	0.007	0.008	1.000
		C57	-0.054 *	0.007	0.000
		B6A	-0.011	0.008	0.996
	B6A	A/J	0.018	0.008	0.218
		C57	-0.043 *	0.007	0.000
		AB6	0.011	0.008	0.996
Premax W/Intraorbital W (D)	A/J	C57	-0.045 *	0.011	0.001
		AB6	-0.037 *	0.012	0.020
		B6A	0.000	0.013	1.000
	C57	A/J	0.045 *	0.011	0.001
		AB6	0.008	0.010	1.000
		B6A	0.046 *	0.011	0.001
	AB6	A/J	0.037 *	0.012	0.020
		C57	-0.008	0.010	1.000
		B6A	0.037 *	0.012	0.022
	B6A	A/J	0.000	0.013	1.000
		C57	-0.046 *	0.011	0.001
		AB6	-0.037 *	0.012	0.022

1 The mean difference is significant at the .05 level.

(continued)

TABLE VIII

Oneway ANOVA of the ratio means

(continued)

Dependent Variable	(I) Strain	(J) Strain	Mean Difference (I-J)	Std. Error	Sig.
Intermolar W/Premax W (E)	A/J	C57	-0.048 *	0.007	0.000
		AB6	-0.035 *	0.008	0.000
		B6A	-0.038 *	0.008	0.000
	C57	A/J	0.048 *	0.007	0.000
		AB6	0.013	0.006	0.268
		B6A	0.009	0.007	1.000
	AB6	A/J	0.035 *	0.008	0.000
		C57	-0.013	0.006	0.268
		B6A	-0.004	0.008	1.000
	B6A	A/J	0.038 *	0.008	0.000
		C57	-0.009	0.007	1.000
		AB6	0.004	0.008	1.000
Palatine Bone W/Premax W (F)	A/J	C57	0.042	0.017	0.101
		AB6	0.020	0.019	1.000
		B6A	0.006	0.020	1.000
	C57	A/J	-0.042	0.017	0.101
		AB6	-0.022	0.016	1.000
		B6A	-0.036	0.017	0.256
	AB6	A/J	-0.020	0.019	1.000
		C57	0.022	0.016	1.000
		B6A	-0.014	0.019	1.000
	B6A	A/J	-0.006	0.020	1.000
		C57	0.036	0.017	0.256
		AB6	0.014	0.019	1.000

1 The mean difference is significant at the .05 level.

Table IX

Discriminant function statistics for measurements

Eigenvalues				
Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	11.214(a)	87.2	87.2	.958
2	1.250(a)	9.7	96.9	.745
3	.402(a)	3.1	100.0	.536

Wilks' Lambda				
Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 3	.026	202.657	21	.000
2 through 3	.317	63.764	12	.000
3	.713	18.759	5	.002

a First 3 canonical discriminant functions were used in the analysis.

TABLE X

Discriminant function structure
for measurements

Standardized Canonical Discriminant Function Coefficients			
	Function		
	1	2	3
Interfissure Distance	.410	-.184	.838
Palatine Fissure L	-1.704	1.270	1.776
Zygomatic Arch L	.633	.538	-2.268
Premax W	.124	-1.234	-1.050
Interorbital W	-.035	-.085	.935
Intermolar W	1.021	.130	.107
Palatine Bone W	.074	.930	-.122
Structure Matrix			
	Function		
	1	2	3
Intermolar W	.581*	.433	.046
Interorbital W	.523*	.223	.084
Palatine Bone W	.504*	.447	-.114
Interfissure Distance	.456*	.225	.235
Premax W	.397*	.256	-.237
Palatine Fissure L	-.020	.517*	-.020
Zygomatic Arch L	.185	.458*	-.217

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions
Variables ordered by absolute size of correlation within function.
* Largest absolute correlation between each variable and any discriminant function

TABLE XI

Classification results[b,c] from the discriminant analysis of the measurements

		Strain	Predicted Group Membership				Total
			A/J	C57	AB6	B6A	
Original	Count	A/J	12	0	0	0	12
		C57	0	24	0	0	24
		AB6	0	0	14	1	15
		B6A	0	0	2	10	12
	%	A/J	100.0	.0	.0	.0	100.0
		C57	.0	100.0	.0	.0	100.0
		AB6	.0	.0	93.3	6.7	100.0
		B6A	.0	.0	16.7	83.3	100.0
Cross-validated[a]	Count	A/J	10	0	0	2	12
		C57	0	24	0	0	24
		AB6	0	0	14	1	15
		B6A	0	0	2	10	12
	%	A/J	83.3	.0	.0	16.7	100.0
		C57	.0	100.0	.0	.0	100.0
		AB6	.0	.0	93.3	6.7	100.0
		B6A	.0	.0	16.7	83.3	100.0

[a]Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

[b]95.2% of original grouped cases correctly classified.

[c]92.1% of cross-validated grouped cases correctly classified.

Table XII

Discriminant function statistics for ratios

Eigenvalues				
Function	Eigenvalue	% of Variance	Cumulative %	Canonical Correlation
1	11.438(a)	92.4	92.4	.959
2	.568(a)	4.6	96.9	.602
3	.378(a)	3.1	100.0	.524

Wilks' Lambda				
Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 3	.037	184.311	18	.000
2 through 3	.463	43.148	10	.000
3	.726	17.964	4	.001

a First 3 canonical discriminant functions were used in the analysis.

TABLE XIII

Discriminant function structure
for ratios

Standardized Canonical Discriminant Function Coefficients			
	Function		
	1	2	3
IFL/Premax W	.443	-.289	.532
Premax W/Palatine Fissure L	1.394	.369	-1.059
Premax W/Zyg Arch L	-.423	-.939	.804
Premax W/Intraorbital W	.387	1.554	-.045
Intermolar W/Premax W	.855	.403	.350
Palatine Bone W/Premax W	-.357	.844	.244
Structure Matrix			
	Function		
	1	2	3
Premax W/Intraorbital W	.168	.489*	-.176
Premax W/Zyg Arch L	.384	-.429*	-.362
Intermolar W/Premax W	.242	.094	.662*
Premax W/Palatine Fissure L	.506	-.344	-.645*
IFL/Premax W	.332	-.014	.643*
Palatine Bone W/Premax W	-.105	-.094	.107*

Pooled within-groups correlations between discriminating variables and standardized canonical discriminant functions
Variables ordered by absolute size of correlation within function.
* Largest absolute correlation between each variable and any discriminant function

TABLE XIV

Classification results[b,c] from the discriminant analysis of the ratios

		Strain	Predicted Group Membership				Total
			A/J	C57	AB6	B6A	
Original	Count	A/J	12	0	0	0	12
		C57	0	24	0	0	24
		AB6	0	0	14	1	15
		B6A	0	0	3	9	12
	%	A/J	100.0	.0	.0	.0	100.0
		C57	.0	100.0	.0	.0	100.0
		AB6	.0	.0	93.3	6.7	100.0
		B6A	.0	.0	25.0	75.0	100.0
Cross-validated (a)	Count	A/J	11	0	0	1	12
		C57	0	24	0	0	24
		AB6	0	0	14	1	15
		B6A	0	0	3	9	12
	%	A/J	91.7	.0	.0	8.3	100.0
		C57	.0	100.0	.0	.0	100.0
		AB6	.0	.0	93.3	6.7	100.0
		B6A	.0	.0	25.0	75.0	100.0

a Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b 93.7% of original grouped cases correctly classified.

c 92.1% of cross-validated grouped cases correctly classified.

DISCUSSION

MEASUREMENTS

Parental Strains

The measurements in this study that most clearly separated the parent strains from each other as well as the F1 strains tended to be the widths. Intermolar width, interorbital width and palatine bone width were each able to significantly separate the A/J and C57 into their own distinct groups (Table VI). The interfissure length was the only length measurement that could equally separate these groups in the same manner. These measurements also contributed significantly to the discriminant analysis functions. The CL-susceptible A/J strain had the narrower width and shorter length of all 4 strains in every instance, while the CL-resistant C57 strain displayed the largest measurements in every instance. None of these results changed when the data were analyzed taking weight into consideration as a covariate (Table VII).

F1 Strains

The B6A was significantly smaller than the AB6 in 4 of the 7 measurements. The trend was for it to be smaller in all 7 of the measurements. This trend seems to indicate that the B6A strain is more similar to its paternal strain than to its maternal strain. This is illustrated well in the groupings of the discriminant analysis (Figure 10). The parental strains are well separated from each other, while the B6A is grouped closer to the A/J than to the C57. Because of the differences found between the two F1 strains, the null hypothesis that there are no differences in craniofacial shape between the F1s is rejected.

The null hypothesis that the shape of the F1 strains will fall somewhere between the shapes of the parental strains is partially rejected, as they were not intermediary in palatine fissure length and zygomatic arch length. The remaining F1 measurements did fall between those of the parental strains.

RATIOS

Parental Strains

Comparing one measurement to another in the form of a ratio is another way of attempting to factor out the innate size difference that may exist between the strains of mice. Two of the ratios were able to completely isolate the A/J from the other 3 strains (Table VIII): the interfissure length to premaxilla width and the intermolar width to premaxilla width. The A/J tended to have a shorter interfissure length, when compared to the width of the premaxilla (i.e., short anterior maxilla and wide premaxilla), and a narrower intermolar width when compared to the premaxilla width (i.e., wide premaxilla and narrow posterior maxilla).

Two different ratios distinctly separated the C57 from the other 3 strains. These were a larger premaxilla width compared to the palatine fissure length and also a larger premaxilla width when compared to the length of the zygomatic arch.

F1 Strains

The F1 strains showed no significant differences between each other in any of the ratios except for the premaxillary width : interorbital width. This is the ratio which also placed the B6A in the same range as the paternal A/J, and the AB6 in the same range as the paternal C57. It is interesting to note, however, that the trend in the values of the

ratios tended to be opposite that of the measurements. Although none reached statistical significance, four of the six ratios placed the two F1's closer to their maternal strain than to their paternal strain.

DISCRIMINANT ANALYSIS

It is not surprising that the results obtained by the discriminant analysis of the ratios revealed similar outcomes to those of the measurements, since this analysis takes all of the measurements or ratios into account in predicting the strain of each specimen, and the ratios are essentially a subset of the measurements. When using the separating power of all the measurements or ratios together, a clearer picture of the separate groupings can be visualized (Figure 10,11). With the measurements, it was mostly the widths and interfissure length of Function 1 that separated the parental strains from each other, leaving the two F1 strains in between the parent strains (Table IX). However, adding the two remaining lengths in Function 2 was able to further separate the two F1 strains from each other. The configuration of the groupings for the ratios is similar to that of the measurements.

Classification results were likewise similar in each of the two discriminant analysis (Table XI, XIV). Over 95% of the cases were correctly classified by strain using the measurement functions, and approximately 94% of the cases were correctly classified using the ratio functions. Mendelian genetics suggest that the two F1 strains would be grouped together, since the genes in each strain are identical. However, the data suggest that the phenotype expressed in the craniofacial shape of the F1's is not the same, and the two strains can clearly be separated.

In the discriminant analysis of the measurements, the B6A group centroid lies closer to the paternal A/J group than to the maternal C57 group, while the AB6 group centroid is approximately equidistance from the two parent strains. Though these features are also present in the ratio analysis, they are not as distinctive.

PREVIOUS STUDIES

Facial Shape

Previous studies have examined the relationship of facial shapes and/or sizes to occurrence of CL in mice.[10-12] Distinct correlations were found. However, the mice examined and measured in those studies were in the embryonic or fetal stages, not adults. When craniofacial measurements of CL-resistant and susceptible strains of mice were compared at the newborn and adult stages, fewer significant differences were found.[13] This is consistent with the findings of this study. The shorter widths and interfissure length found in the A/J strain are also consistent with the findings of previous studies.

Maternal Effect

Many studies[17, 18, 21, 22, 24, 31] have looked at the maternal influence on the incidence of CL in mice. Others have examined size differences[19, 23] and found that the maternal strain did have an influence on the craniofacial size of the F1 strains. However, both of these studies examined the F1's in the embryo stage, not in the adult stage. The results of this study do not support a strong maternal influence persisting through the adult stage, as none of the measurements or ratios exhibited a significant similarity between either of the F1 strains with its maternal strain. There were, however, some

interesting trends. When measurements were compared before controlling for weight, the F1 strains fell closer to their paternal strains than to their maternal strains. When measurements were controlled for weight, however, two of these trends were reversed, and when ratios were compared, all but two of the ratios tended to place the F1 strains closer to their maternal strain, although none were statistically significant. There are at least two possible explanations for this. First, the sample size in this study may be too small to demonstrate any type of maternal effect. Second, there may be different genes expressing themselves in the adult mice than in the embryonic mice. Tanner[35] explains that not all genes are active at birth, and products of some genes can only be expressed in the presence of physiologic circumstances provided later in life. This is, in part, what accounts for the fact that the correlation of an infant's height to that of its parents is only 0.2, yet at 3 years of age it is 0.8. This corroborates with the study by Trasler and Machado[13] in which they noted that the birth weight of the A/J was less than the C57, yet as adults they weighed more.

CLINICAL IMPLICATIONS

The findings of this study may have significant clinical implications. Studies that have examined differences in facial shapes and sizes of parents with CL(P) children have all made the comparisons of the parents after adulthood.[1-3, 32, 36] At this age, many of the craniofacial dimensions that would be related to a CL(P) predisposition may have been outgrown. It may prove interesting to examine measurements obtained from childhood radiographs of parents with CL(P) children and compare to similar radiographs of parents without CL(P) children. This may prove to have more predictive value than comparing the adults.

The present findings may also help explain why maternal effects related to CL(P) in humans have been so difficult to document.[33] It is conceivable that there is an embryonic maternal effect that leads to the formation of CL(P*) in utero*, but the characteristics of facial shape which lead to the cleft formation are outgrown by the age at which the studies are performed.

FUTURE DIRECTIONS

Further research in this area is warranted. There is great power in quantifying the phenotypes of inbred strains of mice. Specific genetic factors can be more easily defined, and increased knowledge of gene expression at various stages in the life cycle would help lead to a better understanding of craniofacial growth and development. This, too, would have great clinical significance. Attaining statistical significance of the measurements examined between the F1 strains in this study may be possible by using larger groups in the samples.

In this study, adult F1s were compared to their parent strains to compare facial shapes. Previous studies have examined either embryonic or fetal F1s, or have looked at different strains of adults without examining F1s at all. Future studies comparing adult mice with their F1 crosses are needed to validate the findings in this study. Other strains of CL-susceptible and –resistant mice should also be compared.

It would also be useful to examine the craniofacial shapes of F1 strains of different ages to their adult parent types. Such comparisons, from embryo through adult, would help quantify when the maternal differences seen in other studies are reversed or outgrown.

There are also other methods of analysis that may be useful in identifying shapes or measurements and quantifying phenotypes that are significantly different between the strains. The Euclidean distance matrix analysis (EDMA) described by Lele and Richtsmeier[37] has been used in numerous studies looking at shape and form differences of the craniofacial complex of many animals.[38-43] This analysis would more easily facilitate the comparison of several additional measurements and shapes of the mouse skull. Perhaps differences would be found in areas that this study, as well as other past studies, have not been examined or compared.

SUMMARY AND CONCLUSIONS

Ward *et al.*[15] reviewed several articles that have found relationships between various craniofacial measurements and the occurrence of cleft lip (CL) in humans. Several other authors have found similar relationships in mice[10-13, 19, 23]. Although it is widely recognized that a relationship exists between oral clefting and facial shape, this relationship is poorly understood[15]. CL occurrence has long been thought the result of a polygenic threshold mode of inheritance[16, 29]. More recent data in studies of CL in mice suggest that genomic imprinting may be a possible explanation for the patterns of inheritance.

Dried skulls of two strains of mice, A/J strain, which has a high spontaneous rate of CL and C57BL/6J (C57) strain, which has virtually no CL, were used in this experiment, as well as two versions of their F1 offspring: AB6F1/J (AB6), in which A/J was the mother and C57 was the father; and B6AF1/J (B6A), with C57 as the mother and A/J as the father. The skulls were digitally photographed and seven craniofacial measurements were made on each group. Six pair of measurements were combined to form ratios which allowed shape to be analyzed as opposed to size only. Measurements and ratios were analyzed using oneway analysis of variance (ANOVA), univariate ANOVA controlling for weight, and discriminant analysis (DA). The null hypothesis (H_o) for this research experiment was that there would be no differences in craniofacial shape between the two F-1 generations of two strains of mice, since both groups would be genetically identical.

Oneway ANOVA showed significant differences ($p<0.05$) between the two parent strains in both the measurements as well as the ratios, with A/J being smallest and C57 largest in all measurements. Univariate ANOVA controlling for weight showed little difference from the oneway ANOVA. DA was able to correctly classify 100% of both parental strains into their correct strain category.

Measurements between the two F1 strains showed fewer significant differences. The B6A strain was significantly smaller than the AB6 in 3 of the 7 measurements, and the tendency was for it to be smaller in all of the measurements. This placed the F1 strains closer to their paternal strain rather than their maternal strain. The only ratio which showed significant difference between the F1's was the premaxillary width to interorbital width in which the B6A exhibited a narrower premaxilla when compared with its interorbital width. This was again more like its paternal strain, though with the remaining 5 ratios, the F1's tended to be closer to their maternal strain. DA was able to correctly classify 89% of the F1's into their correct strain category, indicating significant differences in overall shape between the F1's.

Lack of a strong maternal effect in this study may be do to the age of the mice examined and/or small sample size. Future studies may do well to use the euclidean distance matrix analysis to distinguish additional differences between the 4 strains.

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ABSTRACT

DIFFERENCES IN CRANIOFACIAL SHAPE AMONG

A/J AND C57BL/6J MICE AND THEIR F1 CROSSES

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Several studies have found relationships between various craniofacial measurements and the occurrence of cleft lip (CL) in humans as well as mice. Several modes of inheritance have been proposed, some of which involve a maternal effect. In this experiment, dried skulls of CL-susceptible A/J mice, CL-resistant C57BL/6J (C57) mice and F1 mice of the two reciprocal crosses of the same were measured and compared to ascertain whether differences existed between the two F1 strains, depending on the strain of the mother. AB6/F1J (AB6) have the F1 with A/J as the mother and B6A/F1J (B6A) have C57 as the mother. Digital photographs were measured using digitizing software. Groups of two measurements were combined to form ratios describing specific shapes. Measurements and ratios were analyzed using analysis of variance (ANOVA) and discriminant analysis (DA). Oneway ANOVA showed significant differences ($p<.05$) between the two parent strains with both the measurements as well as the ratios, with A/J being smallest and C57 largest in all measurements. Univariate ANOVA controlling for weight showed little difference from the oneway ANOVA. DA was able to correctly classify 100% of both parental strains into their correct strain category.

Measurements between the two F1 strains showed fewer significant differences. The B6A strain was significantly smaller than the AB6 in 3 of the 7 measurements, and the tendency was for it to be smaller in all of the measurements. This placed the F1 strains closer to their paternal strain rather than their maternal strain. The only ratio which showed significant difference between the F1's was the premaxillary width to interorbital width in which the B6A exhibited a narrower premaxilla when compared with its interorbital width. This was again more like its paternal strain, though with the remaining 5 ratios, the F1's tended to be closer to their maternal strain. DA was able to correctly classify 89% of the F1's into their correct strain category, indicating significant differences in overall shape between the F1's. Lack of a strong maternal effect in this study may be do to the age of the mice examined and/or small sample size. Future studies may do well to use the euclidean distance matrix analysis to distinguish additional differences between the 4 strains.

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University of Iowa, College of Dentistry DDS	Iowa City, IA	8/89-6/93
Advanced Education in General Dentistry Cert	Bolling AFB, DC	7/93-7/94
Indiana University School of Dentistry MSD	Indianapolis, IN	7/01-7/03

Work Experience:

Clinical Research Associates Research Assistant	Provo, UT	10/87-4/89
United States Air Force General Dentist	Yokota AB, Japan	7/98-7/01
	Bitburg AB, Germany	4/95-7/98
	March AFB, CA	8/94-4/95
	Bolling AFB, DC	7/93-7/94

Professional Organizations:

American Dental Association	1989-present
American Association of Orthodontists	2001-present

Presentations:

IADR Poster:	Fracture resistance of teeth with bonded amalgams	
	Acapulco, Mex	April 1991
AADR Poster:	Setting curves of dual-cured cements	
	Boston, MA	March 1992
IADR Poster:	Cusp reinforcement in molars with bonded amalgams	
	Chicago, IL	March 1993
AADR Poster:	Cusp reinforcement in molars with bonded amalgams	
	Seattle, WA	March 1994

Publications:

Fracture resistance of teeth with bonded amalgams; Am J of Dent 1994, Vol 7, No 2, pp 91-94.